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Characterization of Membrane Rafts Isolated From Rat Sertoli Cell Cultures: Caveolin and Flotillin-1 Content

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Membrane rafts from Sertoli cell cultures were isolated as detergent-insoluble glycosphingolipid-enriched (DIG) fractions on the basis of their enriched content of glycosphingolipids and cholesterol and the resulting insolubility in 1% Triton X-100 and their low buoyant density. Because lipid rafts have been implicated in numerous cell functions, including cell signaling and sites for actin/membrane attachment, studies were initiated to characterize Sertoli cell rafts. This study reports the distribution of the raft structural proteins, caveolin and flotillin-1, implicated in raft microdomain organization. Methods employed included the immunoblotting of cell lysates and detergent-insoluble glycosphingolipid-enriched (DIG) fractions, the immunofluorescent microscopy of peritubular myoid cell (PMC) cultures and cryostat-sectioned testis, and the immunohistochemical staining of paraffin-embedded sections following microwave antigen retrieval techniques. Sertoli cells and Sertoli DIG fractions were found to lack the common raft-associated protein, caveolin, a marker protein for caveolae, but they are enriched in the 48-kd protein, flotillin-1, a protein also implicated in raft formation, cell signaling, and cell motility. Since the primary cell contaminant of Sertoli cell cultures is the PMC, these cells, along with spermatogenic cell fraction (SPGC), were also examined for caveolin and flotillin-1 content. The PMCs contained significant concentrations of both caveolin and flotillin-1. PMCs in culture exhibited a punctate caveolin staining pattern at the cell surface characteristic of a caveolar location. These data support the idea that the pinocytotic vesicles observed in PMCs are caveolae. PMCs also show a perinuclear location for caveolin characteristic of a Golgi location. Cryostat sections of rat testis showed a marked concentration of caveolin in the PMCs. The PMC location of caveolin was also confirmed by the immunohistochemical staining of sections from paraffin-embedded rat testis following microwave antigen retrieval techniques. Similar experiments showed a more ubiquitous, stage-specific distribution of flotillin-1 among testicular cell types.

Key words: Peritubular myoid cells, detergent-insoluble glycosphingolipid-enriched fractions

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